The purpose of the present study was to explore the passive and electrically assisted transdermal transport of Sumatriptan succinate (SS) by iontophoresis. For better bioavailability, better patient compliance, and enhanced delivery of SS, an iontophoretic drug delivery system of a thermo sensitive SS gel was formulated using Lutrol F-127. The study was conducted using silver–silver chloride electrodes across hairless pig skin. The effects of pH, pulsed current, polymer concentration, drug concentration, synergistic effect of penetration enhancers on the SS permeation were investigated. Iontophoretic transport of SS was found to increase with increase in the pH of the medium. Viscosity measurements and flux calculations indicated the suitability of the Lutrol gel for transdermal iontophoretic delivery of SS. Anodal pulsed iontophoresis significantly increases the skin permeation of SS at pulsed ratio 1:1 in combination with PEG-400 as penetration enhancers compared with the passive controls.

Keywords: Iontophoresis, Sumatriptan Succinate, Thermosensitive gel.

INTRODUCTION

Sumatriptan succinate (SS) is 3-[2-(dimethylamino) ethyl]-N-methyl-indole-5methanesulfonamide succinate. The free base has a molecular weight of 295.4 and a pKa of 9.63. The succinate salt is freely soluble in water and phosphate buffer, and its molecular weight is 413.5.\(^1\) It is a selective serotonin agonist used to control migraine. In acute treatment of migraine and cluster headaches the usual dose of SS is 100mg to 200 mg by orally as a daily dose in 1hr interval. It undergoes extensive presystemic metabolism in the liver, and therefore its bioavailability is only about 15%. Its \(t_{1/2}\) is 2 hrs and it needs frequent dosing. Sumatriptan itself delays gastric emptying rate and reduces oral absorption. Migraine delays gastric emptying rate and reduces GI absorption. Migraine has symptoms of nausea and vomiting. There is statistically 23% decrease in absorption and delay in absorption (35min) of Sumatriptan from the tablet during migraine as compared to pain free state. It is quite clear that oral delivery of SS encompasses several unattractive features that may be resolved via transdermal administration. Successful iontophoretic delivery would provide enhanced bioavailability and avoid the inconvenience of the intravenous route.

Iontophoresis is a technique that facilitates movement of medication with the application of electric current. The application of constant current is controlled by an electronic device that adjusts the voltage in response to the changes in skin electrical resistance. Charged drug as well as other ions is carried across the skin as a component of induced ion flow. Some important considerations include flux proportionality with respect to applied current density and the presence of ions other than drug (these decrease the efficiency of iontophoretic transport of the drug). Current density up to 0.5 mA/cm\(^2\) is may be considered as tolerable for patients. The onset of action with iontophoretic treatment is rapid, in contrast to hr for passive transdermal delivery. Since drug delivery is proportional to applied current, significant advantages of iontophoresis include the possibility of preprogramming the drug delivery, dose tailoring on an individual basis, or time tailoring in a constant or pulsatile fashion.\(^3\)

Because of the complex nature of the drug delivery, most of the studies related to transdermal iontophoresis are focused on aqueous solutions.\(^4\) Gels are considered to be the most suitable delivery vehicles for iontophoresis, as they can be easily amalgamated with the iontophoretic delivery system and match the contours of the skin. Gels also have other advantages over liquids, such as ease of fabrication into the device, suitability with the electrode design, deformability into skin contours, better occlusion, and better stability. Moreover, the high proportion of water employed in gel formulations can in turn provide an advantageous electroconductive base for clinical use.\(^5\) Lutrol is a...
polyoxypropylene-polyoxyethylene, non-ionic, surface-active block copolymer composed of ~70% ethylene oxide and 30% propylene oxide with an average molecular weight of 115,000 Da. The fact that poloxamer solution (20%-30% w/v in water) forms a reversible gel above 4°C (i.e. solution at low temperature and gel at higher temperature) offers a unique advantage of ease in handling and application. The reversible sol-gel property allows the cool solution to flow onto the skin and spread across it during its transformation to a nonocclusive gel at body temperature. Furthermore, because of the poloxamer solution’s ability to form a hydrogel, it can show good electrical conductivity. In addition, this property can be exploited for refillable unit dose iontophoretic drug delivery systems.

The present study was undertaken to assess the feasibility of delivering SS using Lutrol F-127 as a vehicle for the iontophoretic transdermal delivery. The approach involved checking the drug permeability by passive and iontophoretic transport using an ex vivo hairless pig skin model. The effects of pH, pulse rate, polymer concentration, drug concentration and synergistic effect of penetration enhancers on the SS permeation were examined.

MATERIALS AND METHODOLOGY

Materials: SS (Sun pharma, Barodara, India) and Lutrol F-127 (Wockhardt, Aurangabad, India) were obtained as gift samples. Silver wire (1 mm diameter, 99.9% pure) was purchased from local supplier. Distilled water having a resistivity of more than 18 MΩ was used to prepare aqueous solutions. Other chemicals used in the study were of analytical grade and were purchased from Loba Chem, Research Fine Lab, Mumbai.

Preparation of Electrodes: Silver-silver chloride electrodes were used for their stability and reversibility. The rod-shaped cathode was prepared by dipping the silver wire into the molten silver chloride to form thin and uniform coat. The electrodes were chlorinated by immersing in 0.1 M HCl. The hairless pig skin was completely hydrated, homogeneous, and clear gel. Hence, pig skin was chosen for the permeation studies. Guinea pig which had been given free access to food and water were sacrificed by respiratory paralysis by chloroform immediately before experiment. The hair of the guinea pig skin at dorsal side was removed with hair remover clipper 24 hr before the experiment. The skin was carefully excised; adhering fat and other visceral debris were removed manually. Separated epidermis was washed with saline solution before starting the experiment.

Ex Vivo Permeation Study for optimizing the pH of Donor Medium and pulsatile current: The hairless pig skin was mounted on vertical diffusion cells that were maintained at 37°C ± 1°C using a hot water circulator. The skin was mounted on the diffusion cell with the stratum corneum facing the donor compartment. SS in a concentration of 200 mg/ml was dissolved in prefiltered buffer solutions of pH values 4.2, 6.4, and 7.4. Exactly 1 ml of each SS solution was placed in the donor compartment. The receiver compartment solution for permeation studies was pH 7.4 saline phosphate buffer solution. A constant direct current of 0.5 mA/cm² was applied for iontophoresis using silver–silver chloride electrodes. They prevent electrolysis of water, which may result in pH shifts. Silver wire of 2 cm was used as the anode and silver–silver chloride wire of 4 cm was used as the cathode. The anode was dipped in the donor solution and the cathode in the receptor solution, which was stirred using a Teflon-coated magnetic stirrer at 100 rpm. Passive permeation was tested without application of any current. The same experiment was repeated at pH 7.4 by using a pulsed current having an ON: OFF ratio of 1:1, 1:2, and 1:4.

Preparation of Thermosensitive Gel: Gels were prepared by the cold method. Gels containing 18%, 20%, and 22% w/v of Lutrol F-127 were prepared in phosphate buffer of pH 7.4 to optimize the gelling temperature and viscosity. Gel containing SS and Lutrol F-127 was prepared as follows. Exactly 100 mg/ml of drug was dissolved in a phosphate buffer pH 7.4. It was constantly stirred using a Teflon-coated magnetic stirrer. Exactly 18%, 20%, 22% w/v of Lutrol F-127 was dispersed slowly into this drug solution, and the resulting mixture was then refrigerated at 4°C for 24 hr to obtain a completely hydrated, homogeneous, and clear sol. After that, the sol was removed from refrigerator placed in water bath and temperature was slowly increased until it forms a completely hydrated, homogeneous, and clear gel.

Ex Vivo Permeation Studies Using Thermoreversible Gel: The hairless pig skin with the stratum corneum side facing the donor compartment was mounted on a vertical diffusion cell that was maintained at 37°C ± 1°C using a hot water circulator. Exactly 1 ml of 20% w/v Lutrol F-127 gel containing 200 mg/ml of SS was put into the donor compartment. A pulsatile current of ON: OFF ratio 1:1 was applied for iontophoresis using a silver-silver chloride electrode. Silver wire of 2 cm was used as the anode, and silver-silver chloride wire of 4 cm was used as the cathode. Passive permeation was tested without application of any current. The same experiment was repeated by using Thermoreversible gel containing 5% v/v Dimethylsulphoxide (DMSO), Urea, Polyethylene glycol (PEG) –400 and Tween-80 as penetration enhancers.

Sample Collection and Data Analysis: Exactly 1 ml of the sample was collected after every hour from the side arm of the diffusion cell using a syringe and was replaced with the same volume of pre-warmed (37°C) fresh receptor medium. The
samples collected were sufficiently diluted and tested for the drug content at 280 nm using a UV spectrophotometer (JASCO V-630, Tokyo, Japan). The real steady-state situation was not observed clearly during permeation studies. For this reason the flux ($J_s$) was calculated from the slope of the linear portion of the curve. $Q_s$ is the cumulative amount of drug permeated per cm$^2$ of skin in 8hrs. The enhancement ratio (ER) for the flux was calculated by using Equation 1: $ER = \frac{Iontophoretic Flux}{Passive Flux}$. Permeability coefficient (Kp) was calculated using equation 2: $Kp = \frac{Flux}{Initial amount of drug in donor compartment}$. The percentage of drug ionized was calculated using Equation 3: Percent SS ionized = $100 / (1 + 10^{(pH-pK_a)})$. Statistical analysis of the data was analyzed by one way analysis of variance (ANOVA) followed by Turkey-Kramer test, with the significance level set at 0.05. The data were expressed as mean ± SD. (Graph pad INSTAT 3.01).

**Skin Sensitization Test:** Skin sensitization is a local inflammatory reaction that can appear within minutes to hours after transdermal application and is believed to be initiated by the release of primary cytokines from keratinocytes. The skin sensitization scales were developed by applying sodium lauryl sulphate solution (0.5-5%) on guinea pig. It was determined by visual scoring system depending on degree of erythema; 0 – no erythema, 1-slight erythema (barely perceptible- light pink), 2- moderate erythema (dark pink), 3- moderate to severe erythema (red) and 4- severe erythema (extreme redness). Skin sensitization test was performed on 20% w/v Lutrol F-127 gel containing 200 mg/ml of SS.

**RESULTS AND DISCUSSION**

**Ex Vivo permeation studies of SS to optimize pH of donor medium:** Iontophoresis markedly improved the transdermal permeation of SS. On ionization, Sumatriptan acquires a positive charge. On ionophoresis, the positive charge of the anode pushes positively charged Sumatriptan ions into the skin; this is why its transport across the skin is increased as compared with passive diffusion. As seen in Figure 1, as the pH of the solution is increased, the permeation of SS is increased. With the pH of the donor solution at 7.4, the flux was 33.61 ± 2.61 µg/cm$^2$/hr, while it was only 13.15 ± 1.51 µg/cm$^2$/hr when the donor pH was 4.2 (Table 1). As seen in Equation 2, ionization is a function of the pH of the surrounding medium. Since SS is a very weak acid (pKa 9.16), 100% ionization at pH 7.4 was observed. Therefore, increased ionization and greater repulsion resulted in increased permeation. In addition, it is generally accepted that the stratum corneum possesses a net negative background charge. A pH of 7.4 neutralizes skin’s negative charge and avoids the interruption of skin charge during iontophoretic permeation. Therefore, the remaining studies were performed using pH 7.4 phosphate buffer medium.

**Ex Vivo permeation studies of SS to study the effect of pulsatile current:** Use of continuous direct current may result in skin polarization, which can reduce the efficiency of iontophoretic delivery proportional to the length of direct current application. The buildup of this polarizable current can be overcome by using pulsed direct current that is delivered periodically. Therefore, to further increase the permeation rate and the flux of SS across the skin, pulsed iontophoresis was performed. As seen in Figure 2, the permeation profile of SS at pulsed iontophoresis of ON: OFF pulse ratios 1:2and 1:4 was similar to that of the continuous current (P>0.05). However, the flux was significantly increased at the pulse rate 1:1, (P<0.001) with a flux of 59.47 ± 4.10 ug/cm$^2$/hr (Table 2). The use of pulse current allows the skin to depolarize and return to its initial electric condition when the current phase is put off for a fraction of time. Therefore, the remaining studies were performed using pulse rate 1:1.

**Table 2: Effect of Pulsatile Current on Various Permeation Parameters**

<table>
<thead>
<tr>
<th>Pulsed Ratio(On:Off)</th>
<th>$Q_s$ (µg/cm$^2$/hr)</th>
<th>$J_{ss}$ (µg/ cm$^2$/hr)</th>
<th>Kp</th>
<th>Er</th>
</tr>
</thead>
<tbody>
<tr>
<td>Continuous current</td>
<td>494.80 ± 31.6$^{13}$</td>
<td>33.61 ± 2.61$^{13}$</td>
<td>0.168</td>
<td>1.000</td>
</tr>
<tr>
<td>1:1 ( 1 sec.)</td>
<td>791.02 ± 52.4$^{14}$</td>
<td>59.47 ± 4.10$^{14}$</td>
<td>0.297</td>
<td>1.769</td>
</tr>
<tr>
<td>1:2 ( 1 sec.)</td>
<td>469.29 ± 29.6$^{15}$</td>
<td>35.28 ± 2.35$^{15}$</td>
<td>0.176</td>
<td>1.049</td>
</tr>
<tr>
<td>1:4 ( 1 sec.)</td>
<td>437.55 ± 25.4$^{16}$</td>
<td>33.35 ± 2.52$^{16}$</td>
<td>0.166</td>
<td>0.992</td>
</tr>
</tbody>
</table>

n=3, all readings mean ± S.D. a = statistically significant difference from Continuous Current (P<0.001), b = not statistically significant difference from continuous current (P>0.05).
Development and evaluation of Thermoreversible gel with Lutrol F-127: Gels are clinically acceptable delivery systems for iontophoresis in terms of stability and ease of handling and refilling of iontophoretic patches. Lutrol F-127 (poloxamer 407) is a non-ionic block copolymer that is an intermediate between hydrophilic and hydrophobic polymers. It forms a thermoreversible hydrogel of polyoxy (ethylene oxide)-b poly (propylene oxide)-poly (ethylene oxide). Its 3-dimensional network provides sufficient rigidity, while the highly hydrated microscale environment facilitates mass transfer. Thermoreversible gel has additional advantages over conventional gel. Lutrol F-127 was selected because it forms a thermoreversible gel at the optimized iontophoretic conditions with acceptable viscosity and release characteristics.

The viscosity of gels was determined using a cup and bob viscometer (Brookfield viscometer, model CAP 2000, DV-II). The viscosity of the polymeric solutions containing 18%, 20%, and 22% w/v of Lutrol F-127 in distilled water was determined to assess their gelling characteristics. It demonstrates that an increase in the concentration of Lutrol F-127 increased the gelling property of the gel. At 20% w/v of Lutrol F-127, the gelling property of the gel gradually increased as the temperature increased, with a good viscosity (9224 ± 45cp). Thus, 20% w/v of Lutrol F-127 was the optimum concentration of Lutrol F-127 to produce a gel with sufficient viscosity to hold the formulation in the electrode cavity when the electrode is applied to the skin. These gels were evaluated for viscosity and gelation temperature and evaluation parameters are shown in Table 3.

Table 3: Evaluation Parameters of Lutrol F-127 Gel

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Gelation Temperature(°C)</th>
<th>pH</th>
<th>Viscosity (cp)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>32</td>
<td>5.2 ± 0.2</td>
<td>1440 ± 21</td>
</tr>
<tr>
<td>B</td>
<td>27</td>
<td>5.4 ± 0.2</td>
<td>9224 ± 45</td>
</tr>
<tr>
<td>C</td>
<td>25</td>
<td>5.6 ± 0.1</td>
<td>9986 ± 53</td>
</tr>
</tbody>
</table>

(n=3), All the readings mean ± S.D., * the viscosities at maximum % torque (87-97) and spindle number 64 at 30C.

In general, the gelation temperatures have been considered to be suitable if they are in the range of 25°C to 37°C. If the gelation temperature of a Thermoreversible formulation is lower than 25°C, a gel might be formed at room temperature leading to difficulty in manufacturing, handling, and administering. If the gelation temperature is higher than 37°C, it will not form the gel at the body temperature.

The gelation of Lutrol F-127 vehicle is known to result from the change in micellar number with temperature. With increasing temperature, the number of micelles formed increases as a consequence of the negative coefficient of solubility of block copolymer micelles. Eventually the micelles become so tightly packed that the solution becomes immobile and gel is formed. Recently, Cabana et al. suggested a mechanism of gelation based on micelles packing and entanglements. Also, conformational changes in the orientation of the methyl groups in the side chains of poly (oxypropylene) polymer chains, constituting the core of the micelle, with expulsion of the hydrating water from the micelles will contribute to the gelation phenomenon. Since the gel containing 20% w/v Lutrol F-127 showed good gelling property, viscosity, and conductance, it was considered optimum for iontophoretic drug delivery, and further ex vivo permeation studies were performed on it.

Ex Vivo permeation studies of SS to study effect of drug concentration: This study reveals that increase in drug load (50mg – 100 mg- 125mg) in gel produced no statistically significant increase in the flux (P>0.05) of drug delivered after iontophoresis for 8 hr (Table 4). The steady state flux values obtained herewith were compared by mean of the one way ANOVA followed by Turkey-Kramer test for multiple comparisons.

Ex Vivo permeation studies of SS to study synergistic effect of various penetration enhancers: Passive permeation of drugs across the skin can be increased with transdermal penetration enhancers, as the tightly organised bilayer structure of the skin is weakened. One way to increase drug penetration is to add the penetration enhancer to the drug formulation. Another possibility, is to pre-treat the skin with
the enhancer before drug application. Penetration enhancer and its mechanism of action has been widely studied ex vivo and in vivo. They have been postulated to act by increasing the fluidity of skin lipid, and by forming a fluid phase within the stratum corneum.

In the study various penetration enhancers of 5% v/v were added to the gel. The steady state flux values obtained here were compared by mean of the one way ANOVA followed by Turkey- Kramer test for multiple comparisons. There was no significant increase in the flux when DMSO and Tween-80 were used as penetration enhancers (P > 0.05), but significant increase in the flux was seen with Urea and PEG-400 (P<0.001). Maximum flux of 106.335 ± 7.87 µg/cm²/hr (Table 5) obtained with pulsed iontophoresis in combination with PEG-400. This might be because of the compound was designed as a penetration enhancer for hydrophilic molecules. Its mechanism of action is based on its capability of altering the organization of the lipid structure of the stratum corneum and of increasing the diffusion of the drug into the skin.

**Ex Vivo** permeation studies of SS to study the effect of concentrations of PEG-400: As maximum flux was obtained with pulsed iontophoresis in combination with PEG-400. This study was designed to investigate the effect of various concentrations of PEG-400. When concentration of PEG-400 was increased from 3% to 5% v/v the flux (Table 6) was not statistically increased (P>0.05). But when concentration of PEG-400 was increased to 7% v/v the flux (Table 6) was statistically increased (P<0.001). Further increase in the concentration of PEG-400 from 7 to 10% v/v decreased the flux (Table 6). This may be because of increase in solubility thereby decrease in thermodynamic activity of drug in gel formulation. The steady state flux values obtained here were compared by mean of the one way ANOVA followed by Turkey- Kramer test for multiple comparisons.

**Skin Sensitization Test:** Visual scoring system depending on degree of erythema was developed as fig.6-10. 1gm sample of 20% w/v Lutrol F-127 gel containing 200 mg/ml of SS was applied (12hr) on to the guinea pig skin and erythema was compared with the skin sensitization visual score. No erythema was found within 12hr; this indicates that the developed formulation i.e Thermoreversible gel of SS will not cause any irritation or erythema after application. Thus the developed formulation is suitable for transdermal application.

<p>| Table 5: Synergestic Effect of various Penetration Enhancers on Permeation Parameters |
|-----------------------------------------------|-----------|-------------|-------|-------|</p>
<table>
<thead>
<tr>
<th>Penetration enhancers</th>
<th>Q (5 % v/v)</th>
<th>Jss (µg/cm²)</th>
<th>Kp (µg/cm²/hr)</th>
<th>Er</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ionto (20% gel)</td>
<td>686.45 ± 44.6</td>
<td>60.748 ± 4.12</td>
<td>0.303</td>
<td>1.000</td>
</tr>
<tr>
<td>DMSO7</td>
<td>66.45 ± 52.2</td>
<td>65.67 ± 4.1</td>
<td>0.328</td>
<td>1.081</td>
</tr>
<tr>
<td>Urea</td>
<td>818.89 ± 56.4</td>
<td>87.045 ± 5.97</td>
<td>0.435</td>
<td>1.432</td>
</tr>
<tr>
<td>PEG-400</td>
<td>878.34 ± 62.3</td>
<td>106.335 ± 7.87</td>
<td>0.531</td>
<td>1.750</td>
</tr>
<tr>
<td>Tween-80</td>
<td>690.75 ± 44.8</td>
<td>64.59 ± 4.83</td>
<td>0.322</td>
<td>1.063</td>
</tr>
</tbody>
</table>

n=3, all readings mean ± S.D. a = statistically significant difference from ionto 20% gel (P<0.01), b = statistically not significant difference from passive 20% gel (P>0.05).

<table>
<thead>
<tr>
<th>Table 6: Effect of deferent Concentration of PEG-400 on Various Permeation Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEG-400</td>
</tr>
<tr>
<td>------------------------</td>
</tr>
<tr>
<td>Ionto (20% gel)</td>
</tr>
<tr>
<td>PEG-400 (3%)</td>
</tr>
<tr>
<td>PEG-400 (5%)</td>
</tr>
<tr>
<td>PEG-400 (7%)</td>
</tr>
<tr>
<td>PEG-400 (10%)</td>
</tr>
</tbody>
</table>

n=3, all readings mean ± S.D. a = statistically significant difference from ionto 20% gel (P<0.01), b = statistically not significant difference from passive 20% gel (P>0.05).
Fig. 6: Score 0 – No Erythema

Fig. 7: Score 1-Slight Erythema (Barely Perceptible- Light Pink)

Fig. 8: Score 2- Moderate Erythema (Dark Pink)

Fig. 9: Score 3 - Moderate to Severe Erythema (Red)

Fig. 10: Score 4 - Severe Erythema (Extreme Redness)
CONCLUSION

Lutrol F-127 could be used to formulate a thermosensitive gel for iontophoresis that will gel upon application to skin. Because of neutralization of skin charges and complete ionization of SS, permeation was significantly enhanced at pH 7.4. Due of periodic depolarization of skin, pulsed iontophoresis with penetration enhancer PEG-400 showed better flux enhancement, and iontophoretic transport of SS was almost ten times as much as for passive transport. The present study demonstrated the feasibility of SS transdermal transport through Lutrol F-127 gel by iontophoresis. Further In vivo studies will be required to support in vitro conclusions and develop In vitro - In vivo correlations.

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